Characterizing the role of IgG antibodies in anaphylaxis

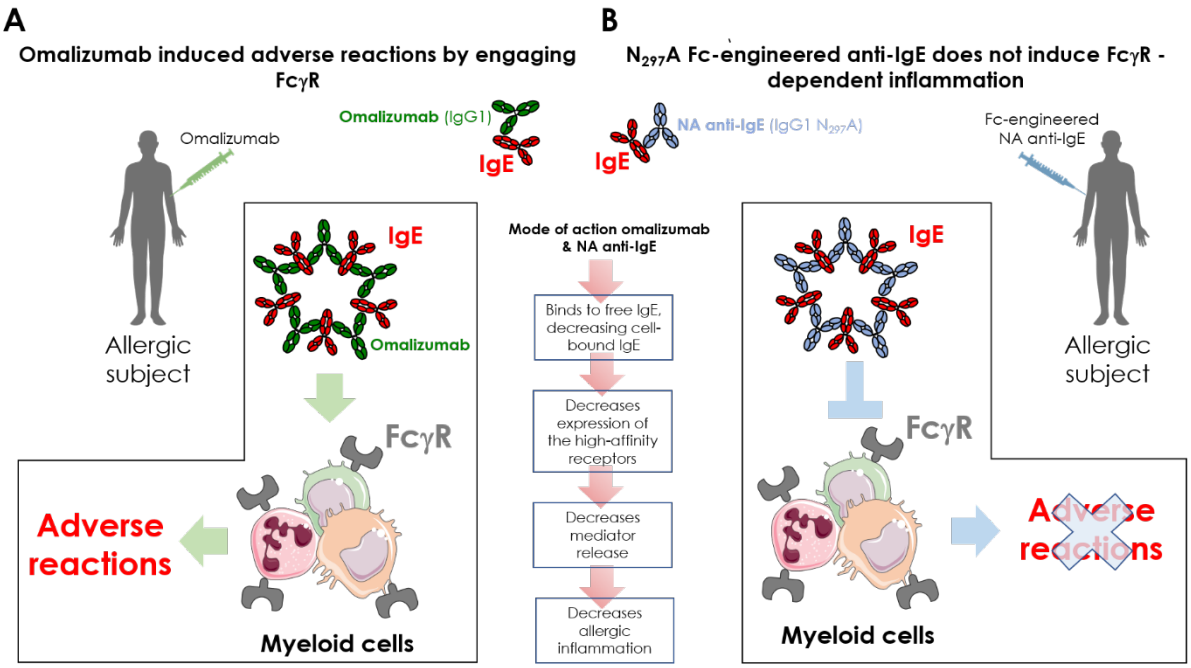
Allergies can affect up to 20% of the population and are increasing worldwide with unprecedent complexity and severity. Allergies are acquired unbalanced immune response to innocuous elements, called allergens, such as pollen or food. In the case of an allergic (sensitized) subject, reactions can be elicited within minutes to hours after the contact with the allergen. Reactions include mostly local-specific reactions, such as rash or hives, itchiness and, runny nose. Yet, in some cases, symptoms may expand to life-threatening circulatory collapse: systemic shock or anaphylaxis. Anaphylaxis is classically described to rely on the 'classical' pathway, in which allergen-specific IgE antibodies bind to the high-affinity receptor (FcεRI) expressed on mast cells and basophils[1]; upon allergen exposure, such cells release mediators responsible for systemic shock. The main focus of my thesis was to question the paradigm of the sole contribution of the classical pathway in anaphylaxis by using innovative mouse approaches.

Several reports in mice indicate that mouse IgG antibodies can also trigger anaphylaxis, but the identity of the effector cell(s) mediating this reaction is still a matter of debate. Most of the anaphylaxis models employ non-physiological adjuvants during the sensitization phase to elicit a robust antibody response. Since, many of these mouse models can be induced with little or no contribution of mast cells and IgE. We evaluated the contribution of IgE and IgG receptors (Fc receptors), effector cells and mediators in an adjuvant-free mouse model of active systemic anaphylaxis. In this model, we observed a modest contribution of the 'classical' pathway mediated by IgE, FcεRI, mast cells and histamine. Anaphylaxis was mediated mainly by an 'alternative' pathway driven by IgG, its receptor FcγRIII, macrophages and platelet-activating factor (PAF)[2].

To assess the clinical relevance of our findings, we studied whether and how human IgG could trigger anaphylaxis. Most therapeutic antibodies used in the clinics are human or humanized IgG. Some of these monoclonal antibodies (mAbs), including the anti-IgE mAb omalizumab (an IgG1 mAb), have been reported to induce anaphylaxis in some patients. To evaluate whether omalizumab-induced anaphylaxis rely on the engagement of human IgG receptors (FcγRs), representing an example of the 'alternative' pathway of anaphylaxis in humans. We performed ex-vivo tests that revealed that omalizumab forms immune complexes (ICs) with IgE, which can engage FcγRs that have the potential to activate human neutrophils. Using a unique humanized mouse model developed in our laboratory, which expresses all FcγRs in place of the mouse IgG receptors (Figure A)[3]. We could show that such ICs induce both skin inflammation and systemic anaphylaxis (most common and most extreme reaction observed in patients receiving omalizumab, respectively). Since IgG binds their FcγRs via the Fc portion of the antibody, we engineered an Fc-variant of omalizumab that is mutated in a glycosylation site (N297) necessary for FcγRs binding. This single mutation was able to significantly reduce both local and systemic adverse reactions induced by omalizumab, without perturbing its ability to block IgE (Figure B). Therefore, we propose this Fc-engineered mAb as an alternative to omalizumab in patients with high levels of IgE,

history of anaphylaxis or other adverse reactions to the drug. A patent application[4] protects the potential applicability of our findings.

In summary, the first part of my thesis further clarified the mutual contribution of classical (IgE-dependent) and alternative (IgG-dependent) pathway using a mouse model of anaphylaxis. Furthermore, we showed the potential contribution of specific IgGs in monoclonal antibody-induced anaphylaxis, and developed an Fc-engineered version of omalizumab with significantly reduced effector function and therefore less adverse reactions, while still able to block IgE.



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